Introduction of the AOP together with brief description of OECD-sponsored AOP Knowledge base (AOP-KB)

Ivana VINKOVIĆ VRČEK
Scientific Adviser
Institute for Medical Research and Occupational Health, Zagreb (Croatia)

Webinar on nanotechnology risk-assessment method AOP
05 06 2020

This project has received funding from the European Union's Horizon 2020 programme: grant agreement 814425.

Adverse Outcome Pathway (AOP)

- emerged in 1980's from the field of ecotoxicology
- to address uncertainty in risk assessment for an increasing number of chemicals and endpoints
- to utilize the quantitative structure activity relationship (QSAR), biomarkers, and other types of mechanistic data
- basic premise - toxicity results from biologic failure initiated by the interaction of a chemical with some biomolecule in the body
- allows for the integration of all types of information at different levels of biological organization, from molecular to population level

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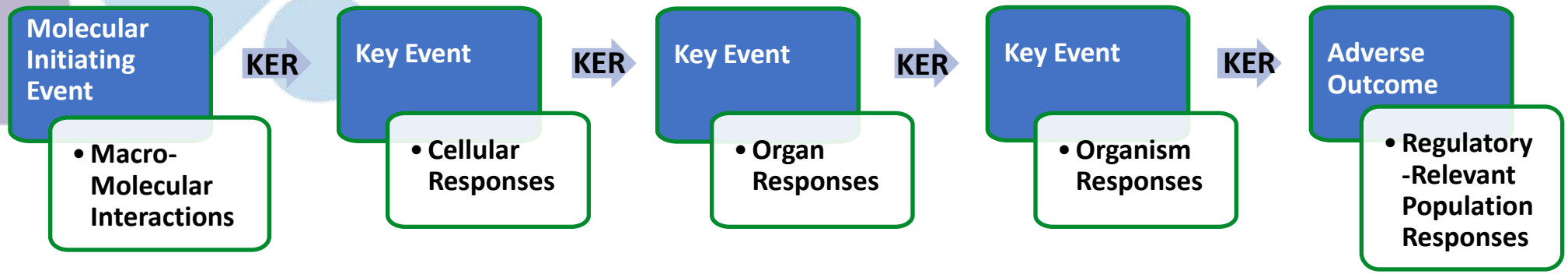
Institute for Medical Research and Occupational Health, Zagreb (Croatia)

AOP concept

AOP consists of 4 primary components:

- Molecular Initiating Event (MIE)
- Key Event (KE)
- Key Event Relationship (KER)
- Adverse Outcome (AO)

AOP describes a sequence of events



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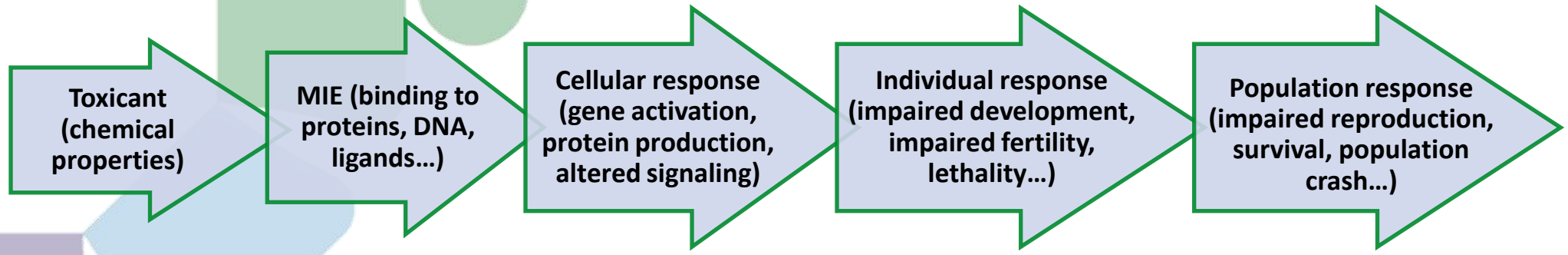
AOP development

Linking upstream molecular changes to adverse outcomes:



Main AOP characteristics:

(1) not chemical specific,
(2) modular structures (consisting of KEs and KERs),
(3) pragmatic units of development and evaluation,
(4) AOP networks are the functional unit of prediction,
(5) living documents (continuously updated and never finished).

Source: C. Willet. In: Alternatives to Animal Testing. Ed.: H. Kojima et al. Pp. 83-90.2019. DOI: 10.1007/978-981-13-2447-5_11

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AOP development strategy

- Top-down AOP development
  (apical AO of interest → to connect it with an MIE)
- Bottom-up AOP development
  (well-defined MIE → higher levels of biological organization)
- Middle-out AOP development
  (observable KE → connections to mechanisms)
- AOP development from a case study
  (well-defined sequence of biological events for a single chemical → generalization to others)
- AOP development by analogy
  (AOP defined for particular animal model → alternative KEs and KERs for other organisms)
- AOP development from data-mining
  (high content and/or high-throughput data sets → data mining to infer relationships between KEs)

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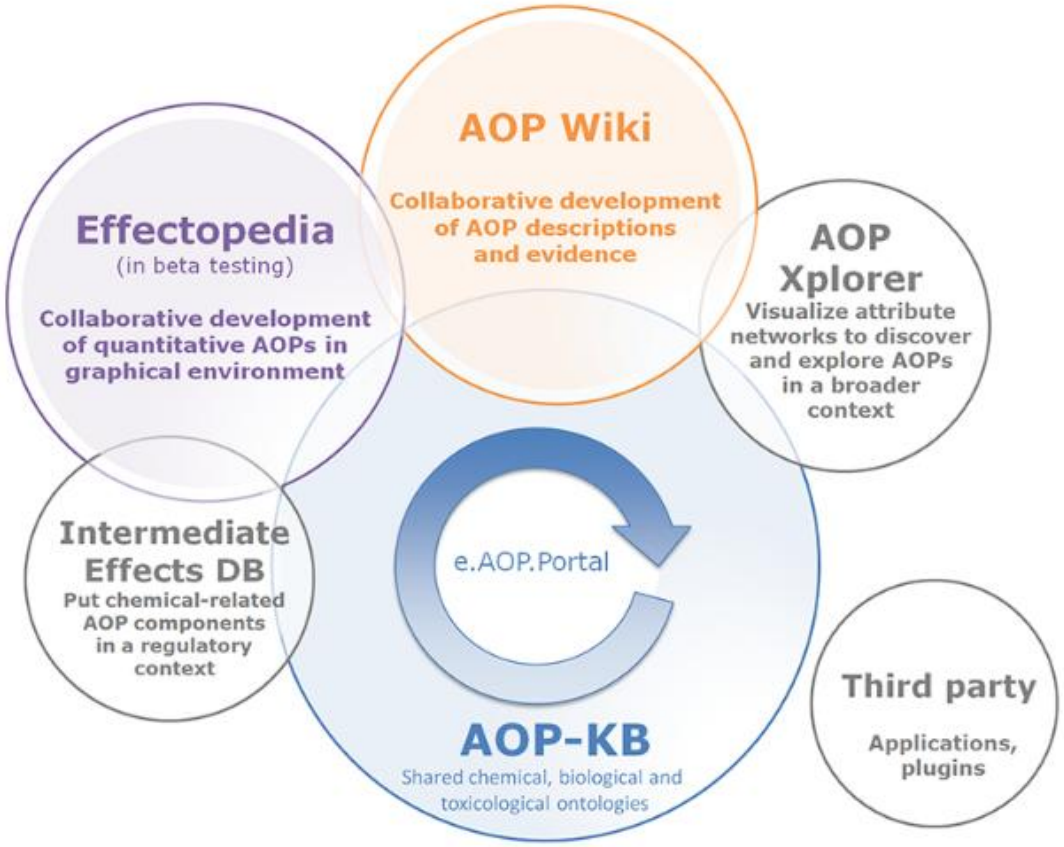
Institute for Medical Research and Occupational Health, Zagreb (Croatia)

AOP Knowledge Base

RISK GONE

- OECD created AOP framework (use of molecular- and cell-based information to inform regulatory decisions);

- AOP Knowledge Base (AOP-KB)
  - developed by the OECD Extended Advisory Group on Molecular Screening and Toxicogenomics (EAG MST)
  - implemented by the European Commission's Joint Research Centre (JRC) and the US Environmental Protection Agency (US-EPA)



Copied from the https://aopkb.oecd.org/index.html

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Institute for Medical Research and Occupational Health, Zagreb (Croatia)
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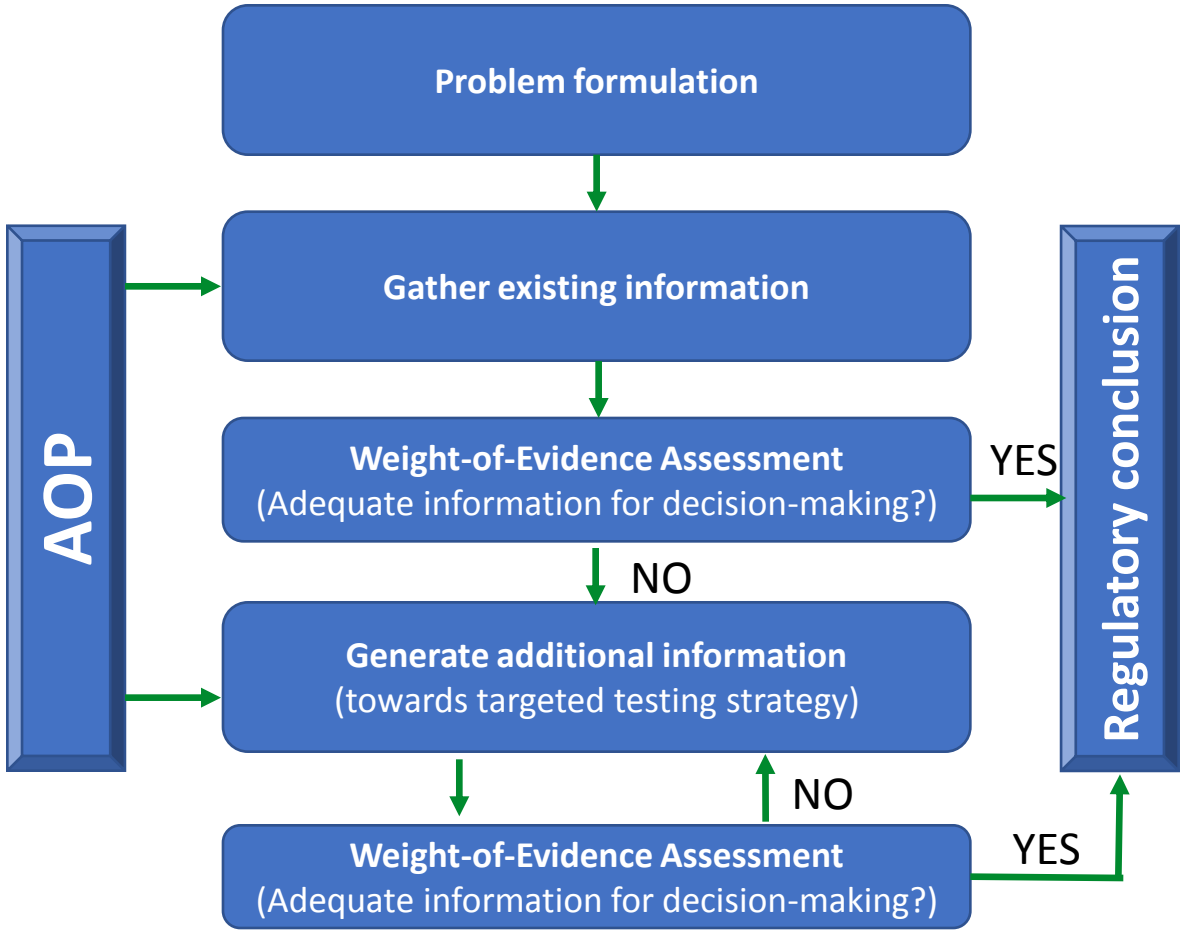
AOP – Regulatory Relevance

AOP - the basis for an integrated approach to testing and assessment (IATA) or an integrated testing strategy (ITS).

Potential uses:

1) supporting chemical category formation and "read-across" (predicting the toxicity of one chemical based on results from a related chemical),
2) priority setting for further testing,
3) hazard identification
4) classification and labeling,
5) risk assessment.



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- M. Sachana and E. Leinala. Applied In Vitro Toxicology 2017, 3(3), 227-233. DOI: 10.1089/aivt.2017.0013
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- H.J. Clewell, J.W. Yager, T.B. Greene, P.R. Gentry. Journal of Toxicology and Environmental Health, Part A 2018, 81(18), 893-912. DOI: 10.1080/15287394.2018.1500326

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RISK GONE

THANK YOU!

Ivana VINKOVIĆ VRČEK
Scientific Adviser
Institute for Medical Research and Occupational Health, Zagreb, Croatia
ivinkovic@imi.hr

www.riskgone.eu | riskgone@nilu.no

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SESSION 2:AOPs FOR NANOMATERIALS

Peter HOET, Professor
Sivakumar MURUGADOSS, Research Associate
KU Leuven

AOP Webinar
Place, 05 06 2020

This project has received funding from the European Union's Horizon 2020 programme: grant agreement 814425.

RISK GONE

Overview

Part 1: Sytematic search to identify AOPs and potential (molecular) initiating events (MIE)/key events (KE) reported for nanomaterials

Part 2: Identifying existing AOPs in AOP wiki using identified MIE/KE

Part 3: Generating testable AOPs for nanomaterials

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Part 1: A systematic literature search to identify AOPs for nanomaterials

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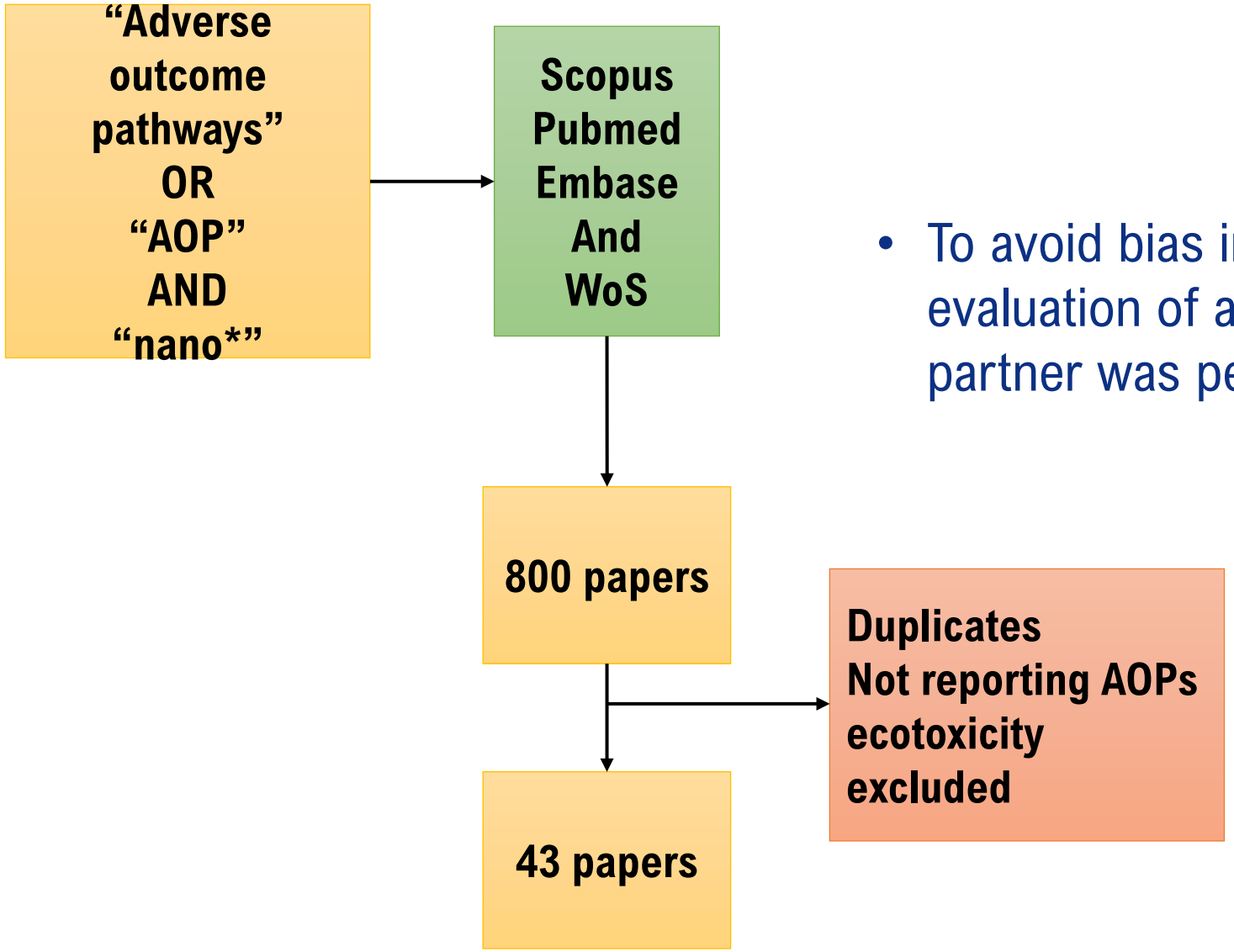
SYSTEMATIC REVIEW ON AOPs FOR NANOMATERIALS



- To avoid bias in selection - Parallel evaluation of articles by another partner was performed

DATA EXTRACTION

- A basic AOP template was prepared and used for data extraction

No.	Nanomaterials	Cell type	Animal	Important phy-chem characteristics	Exposure condition		Molecular initiating event	key event 1	key event 2	key event 3	key event 4	Adverse outcome	
	(stressor)	*in vitro*	*in vivo*		conc	duration		organelle response	Cellular response	Tissue response	organ response	Organism level	population level

DATA EXTRACTION

Nikota et al 2017

Nanomaterials (stressor)	Animal *in vivo*	Important phy-chem characteristics	Exposure condition		Molecular initiating event	key event 1	key event 2	key event 3	key event 4	Adverse outcome	
			conc	duration		organelle response	Cellular response	Tissue response	organ response	Organism level	population level
MWCNTS	C57BL/6 mice	L-3.86 µm and D ± 13.4 nm	162 µg in a 50 µl	1 and days 28 exposure.	Cellular sensing	induction of cytokines CXCL1, IL-6, and IL-12	Persistent inflammation neutrophils persistent increase	increase of pro-fibrotic genes CCL2, OPN (osteopontin) and TGF-β	Excessive ECM increased collagen deposition Fibroblast proliferation increased vimentin signal	Lung Fibrosis	

DATA EXTRACTION

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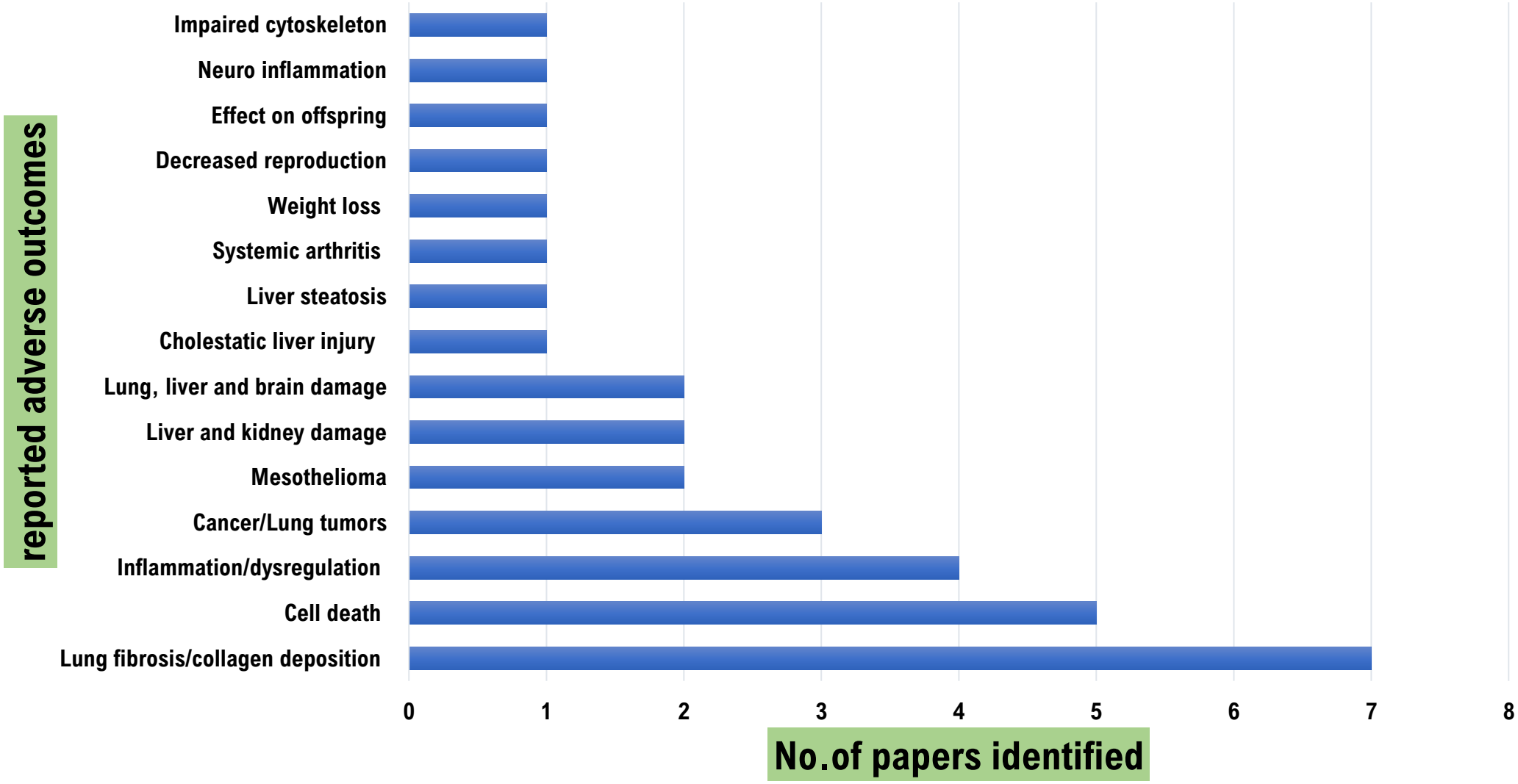
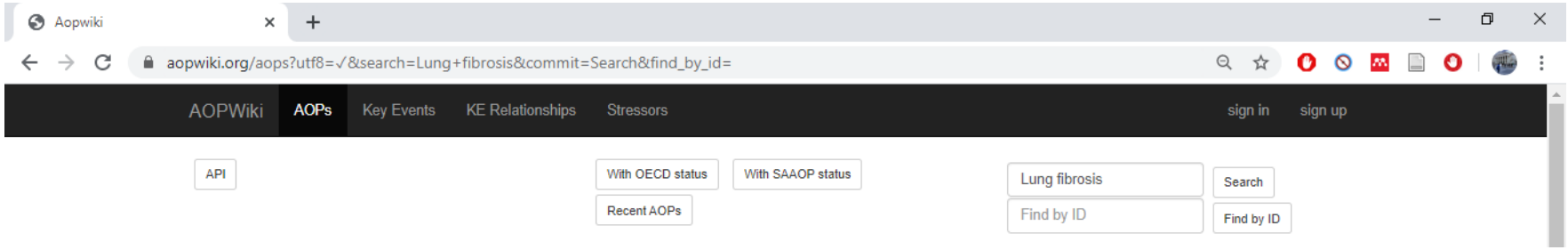
Yang et al 2010

Nanomaterials (stressor)	Animal *in vivo*	Important phy-chem characteristics	Exposure condition		Molecular initiating event	key event 1	key event 2	key event 3	key event 4	Adverse outcome	
			conc	duration		organelle response	Cellular response	Tissue response	organ response	Organism level	population level
CuNP	male Wistar rats	nominal size 25 nm; average size 90 nm after dispersing in 1% hydroxypropylmethylcellulose solution (by DLS and AFM); specific surface area 6.92 m2/g; dissolution after 30 min sonication 0.014 ± 0.002%	100 and 200 mg/kg	oral gavage for 5 consecutive days, animals sacrificed 24h after last application	ROS formation	upregulation of stress-response genes (HMOX1, CYS1A1, NQO1, A2M, AKR1B8, GPX1, HSD17B2) altrered transcription of genes related to major metabolic pathways (glycolysis and gluconeogenesis, mitochondrial fatty acid betaoxidation, fatty acid metabolism, lipid biosynthesis, cholesterol synthesis, steroid synthesis, and the urea cycle) activation of MAPK signalling cascade alteration of Jak-STAT and insulin signalling pathways	oxidative stress response (repair intracellular damage or remove the toxicant) response to ATP depletion (limiting energy-consuming pathways and increasing ATP synthesis)	moderate histopathological changes in liver (hepatocytic necrosis) at 200 mg/kg	increase in ALT, AST, total serum triglycerides, billirubin and bile acid, decrease in ALP and total cholesterol	significant weight loss	

CONSOLIDATION OF ADVERSE OUTCOMES (AOs)



IDENTIFIED AOs – STATUS IN AOP WIKI?



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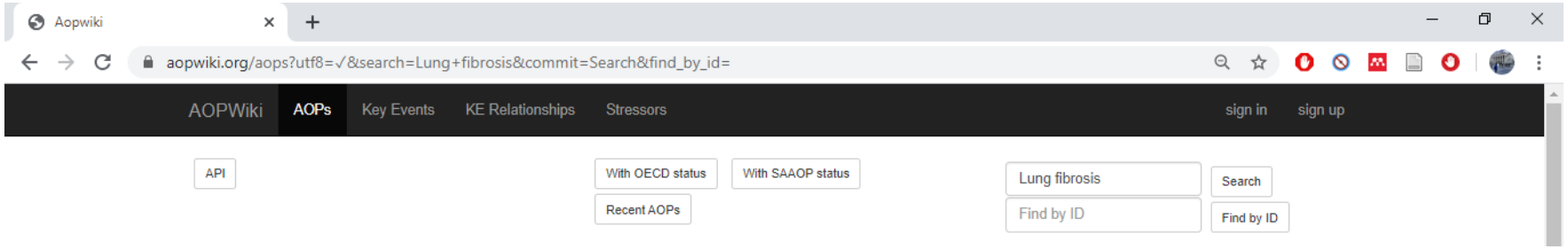

Particle type	*AOs*	*Identified as*	*Link to the AOP wiki source*
CNTs, graphenes and CB	Lung fibrosis/collagen deposition	AO	https://aopwiki.org/aops/206
CNTs and multiNPs	Cell death/apoptosis	AO	https://aopwiki.org/aops/205
C,Ag,ZnO and CeO and TiO2	Cancer/Lung tumors	AO	https://aopwiki.org/aops/139
CNTs	Mesothelioma	AO	https://aopwiki.org/aops/171
TiO2,CeO2 and Ag	Death	AO	https://aopwiki.org/aops/96
CuO	Decreased body weight	AO	https://aopwiki.org/aops/6
Ag	Decreased reproduction and increased mortality	AO	https://aopwiki.org/aops/290
Fullerene,CNTs TiO2 and PM2.5	Effect on offspring	AO	https://aopwiki.org/aops/42
Mesoporous SiO2	Cholestatic liver injury	AO	https://aopwiki.org/aops/27
ZnO	Liver steatosis	AO	https://aopwiki.org/aops/34
SiO2, Fe2O3,CoO,REO,Ag,ZnO and crystalline silica	Inflammation/dysregulation	KE	https://aopwiki.org/aops/303
CNTs	Neuro inflammation	KE	https://aopwiki.org/aops/17
Ag and GO	Impaired cytoskeleton	KE	https://aopwiki.org/aops/70
GdO, MnO and CuO	Liver and kidney damage	N/A	
CNTs	Systemic arthritis	N/A	

- Similar adverse outcomes also found in AOP wiki

IDENTIFICATION OF MIE/KE

Adverse outcomes (AO)	Models	Molecular Initiating event (or first event reported in the study)
Lung fibrosis	in vivo	CNT cellular interaction
Mesothelioma	in vivo	CNT cellular/tissue interaction
Lung fibrosis	in vivo	CNT cellular/tissue interaction
Cardiac dysfunction in fetuses/offspring	in vivo	CNT cellular/tissue interaction
Cell death and DNA repair impairment	in vitro	CNT cellular interaction
Pulmonary inflammation and fibrosis	in vivo	CNT cellular/tissue interaction
Mesothelioma	in vivo	CNT cellular/tissue interaction
Lung fibrosis	in vivo	CNT cellular/tissue interaction
Antioxidant defense, Inflammation, impaired men	in vitro	NP direct interaction with biomolecules/membranes
Persistent lung inflammation (proposed)	in vitro and in vivo	surface silanol disorganization and Membrinolysis
Death and cancer progression	in vitro	ROS formation
weight loss	in vivo	Free radical (ROS) formation
Liver and brain damage	in vitro	ROS formation and dopamine receptor antagonist
Apoptosis	in vitro	ROS formation/amino acid and Glycerophosphocholine accumulation
Cell death	in vitro	ROS formation?
Apoptosis	in vitro	ROS formation
Liver and kidney damage	in vivo	MDA fomation and mitochondrial dysfunction
Lung fibrosis	in vivo and in vitro	Lysosome injury
Cell death	in vitro	Lysosomal acidification
Collagen deposition	in vitro and in vivo	Lysosome injury
Lung fibrosis	in vitro	Genotoxicity
Decreased reproduction and increased mortality	in vivo	Apoptotic stimuli/ROS formation/DNA damage
Impaired cytoskeleton	in vitro	DNA methylation?
Cancer	in vitro	DNA methylation?
Arthritis	in vivo and in vitro	Induction of IL1β and TNFα (TNFα and IL6 in invivo)
Cholestatic Liver injury	in vitro	induction of IL1 and TNFα/BSEP- inhibition
Systemic inflammation and anemia	in vivo	Induction of IL6
Systemic (neuro) inflammation	in vivo	inflammation in the lung?
Kidney damage	in vivo	interuption of calcium homeostatis
Liver and Lung damage	in vitro	altered signalling pathways associated with cyotoxicity ?
Systemic shortage of lipid or hepatic steatosis	in vivo	altered expression of lipid systhesis liver growth factors and apoptotic genes?
Immune system dysregulation	in vitro	activation of intracellular pattern recognition receptors
Lung tumors	in vivo	lung overload?

IDENTIFICATION OF MIE/KE

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Lung fibrosis	in vivo	CNT cellular interaction
Mesothelioma	in vivo	CNT cellular/tissue interaction
Lung fibrosis	in vivo	CNT cellular/tissue interaction
Cardiac dysfunction in fetuses/offspring	in vivo	CNT cellular/tissue interaction
Cell death and DNA repair impairment	in vitro	CNT cellular interaction
Pulmonary inflammation and fibrosis	in vivo	CNT cellular/tissue interaction
Mesothelioma	in vivo	CNT cellular/tissue interaction
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Cell death	in vitro	ROS formation?
Apoptosis	in vitro	ROS formation
Liver and kidney damage	in vivo	MDA fomation and mitochondrial dysfunction
Lung fibrosis	in vivo and in vitro	Lysosome injury
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Collagen deposition	in vitro and in vivo	Lysosome injury
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Systemic shortage of lipid or hepatic steatosis	in vivo	altered expression of lipid systhesis liver growth factors and apoptotic genes?
Immune system dysregulation	in vitro	activation of intracellular pattern recognition receptors
Lung tumors	in vivo	lung overload?

CNT Cellular interaction

ROS formation

Lysosome injury

DNA damage

Inflammation

SUMMARY: PART 1

Adverse outcomes reported for nanomaterials

- Lung based AOs (Lung fibrosis, lung cancer, mesothelioma)
- Liver based AOs (Liver steatosis, liver damage and cholestatic liver injury)

Potential MIE/KE for nanomaterials

- CNT cellular interaction
- Lysosome injury
- ROS formation
- DNA damage

Part 2: Search for AOPs in AOP wiki using identified MIE/KE

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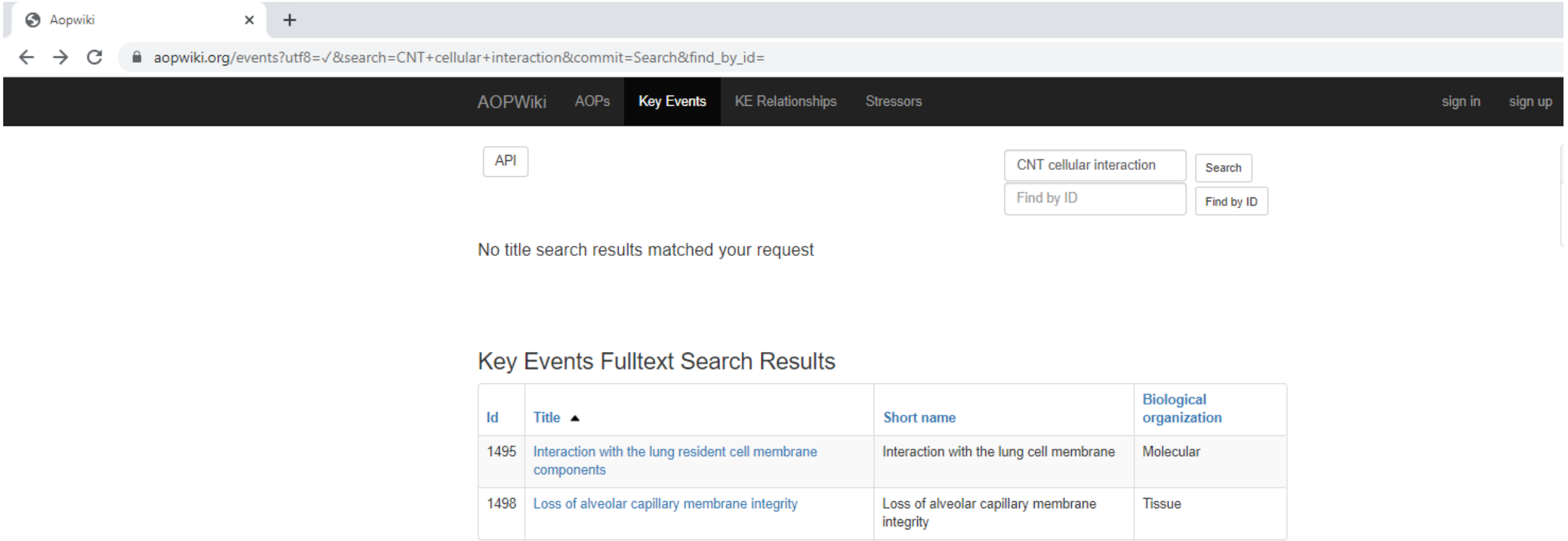
KEYWORD SEARCH IN AOP WIKI



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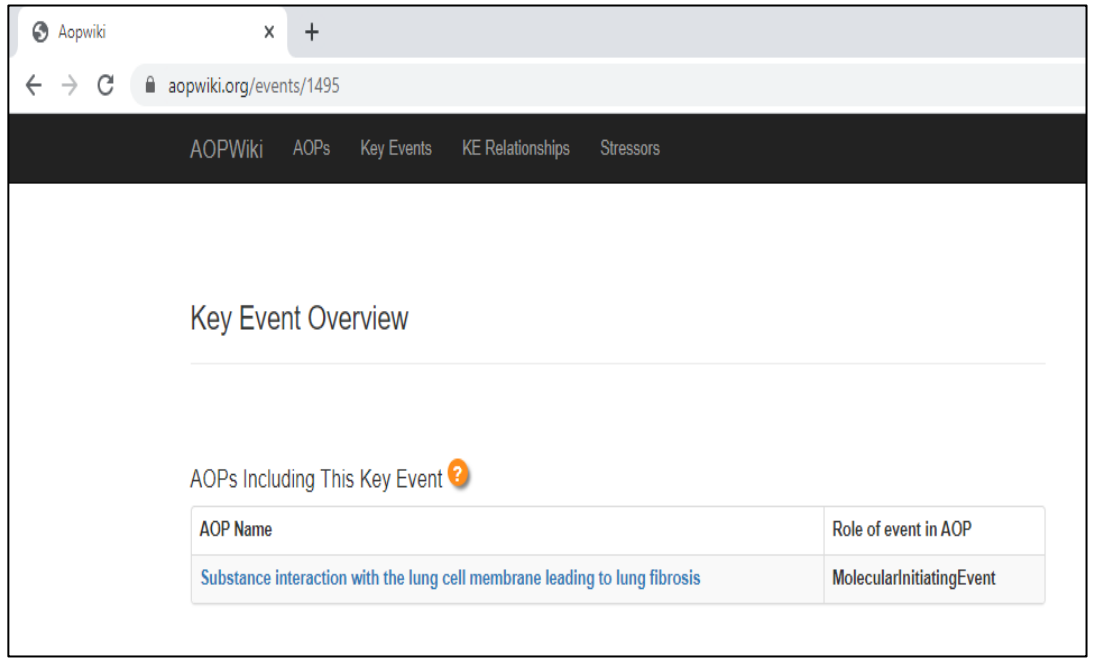
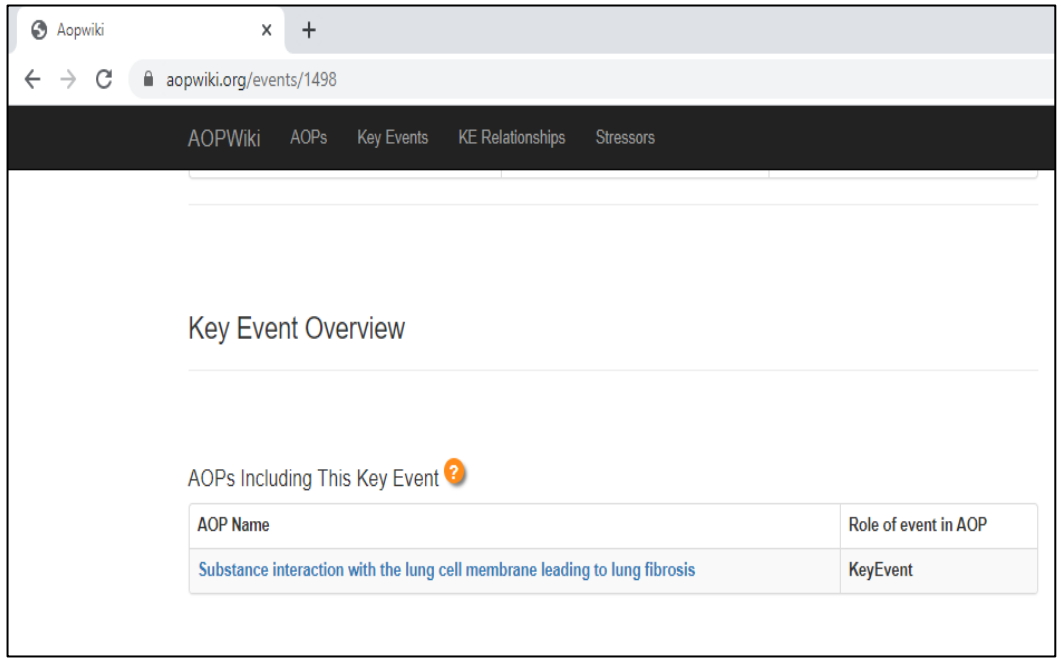
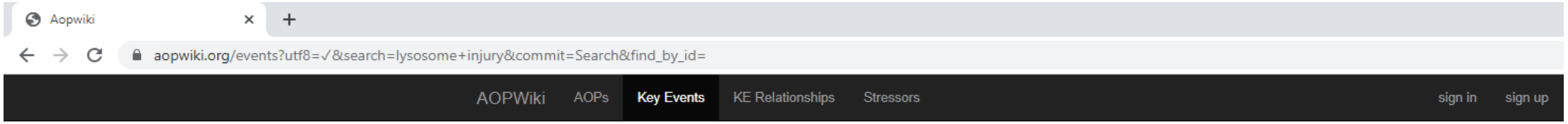




Same AOP!

KEYWORD SEARCH IN AOP WIKI



API

lysosome injury | Search

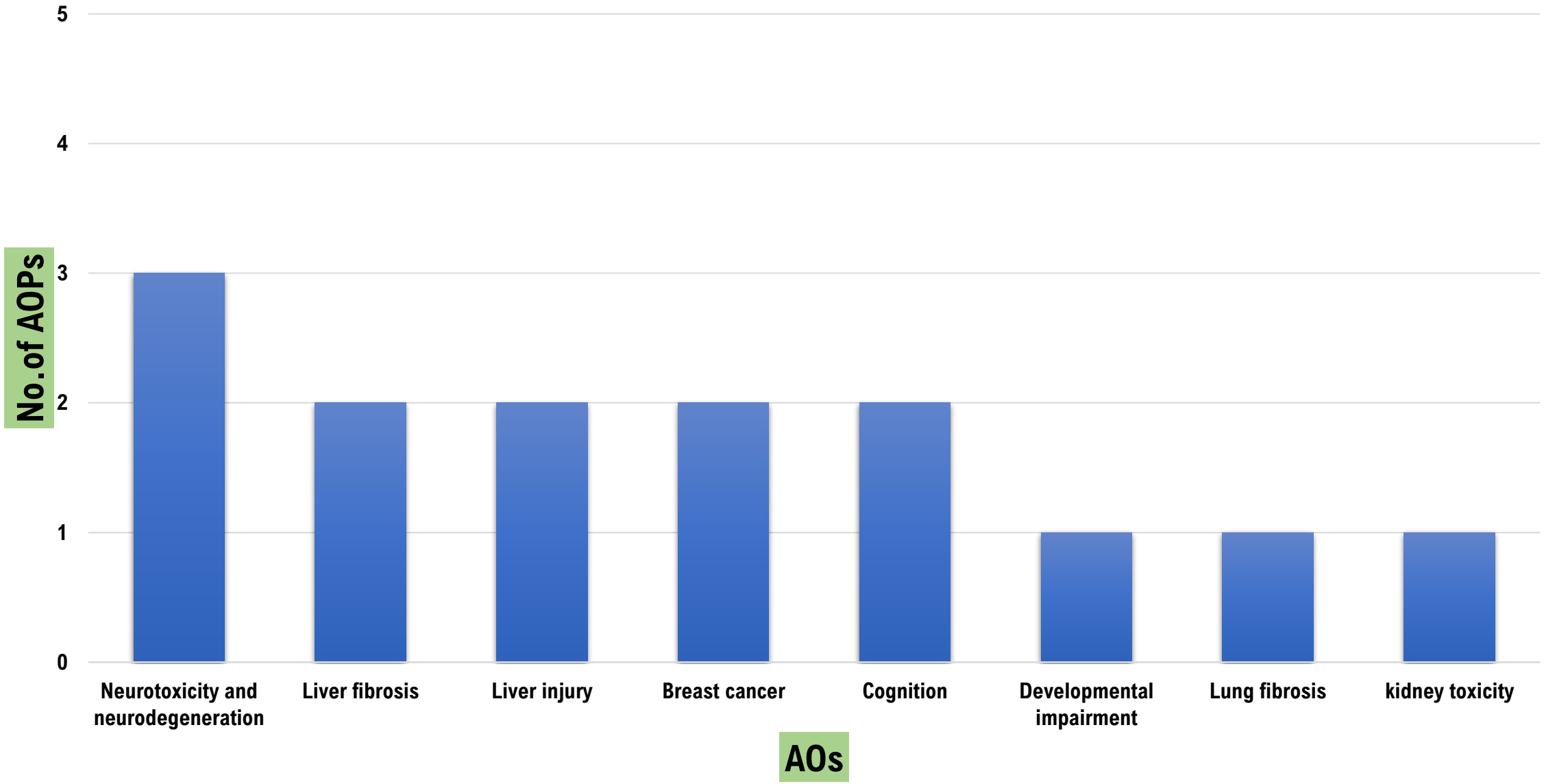
Find by ID | Find by ID

No title search results matched your request

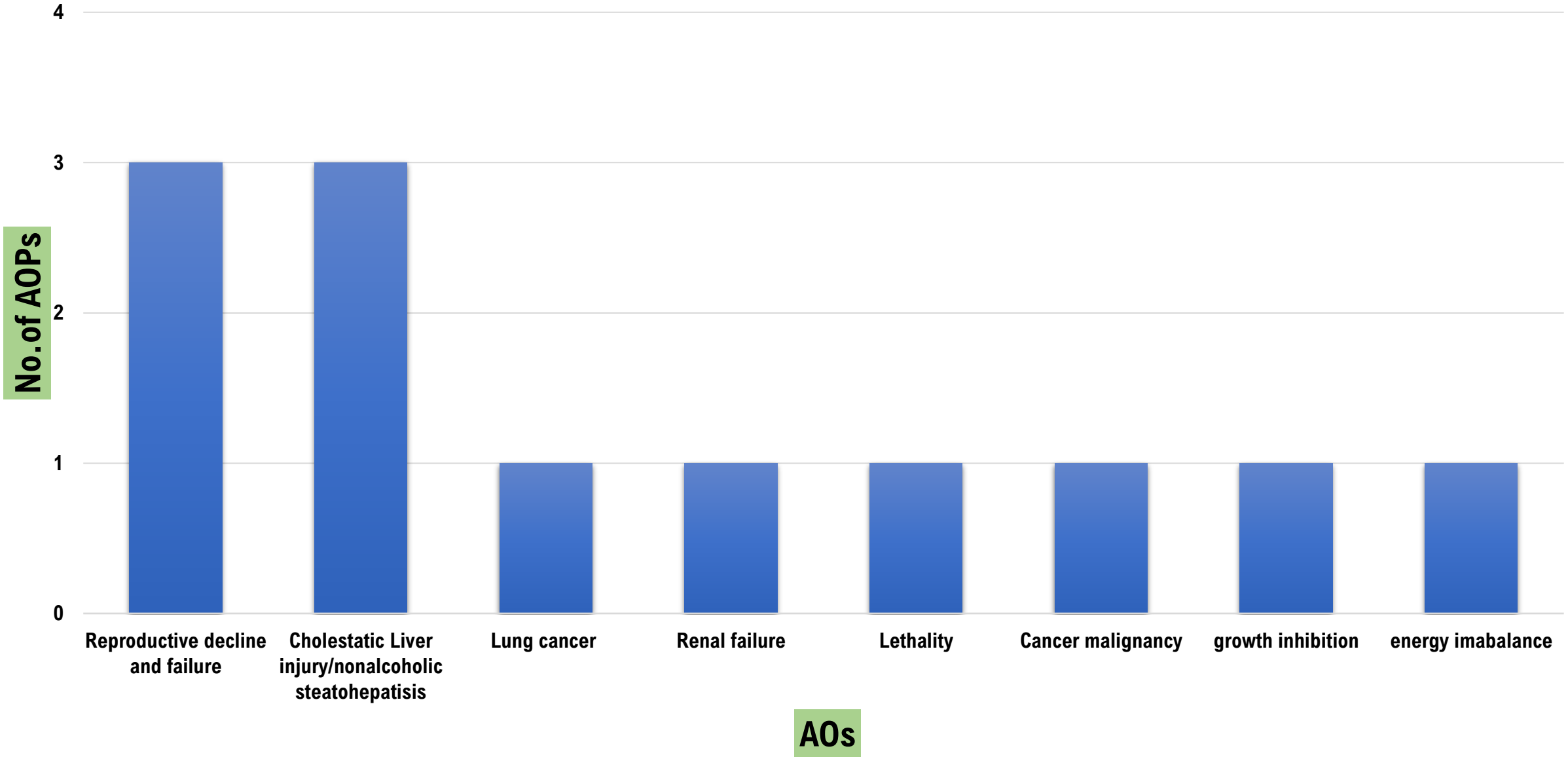
Key Events Fulltext Search Results

Id	Title ▲	Short name	Biological organization
898	Disruption, Lysosome	Disruption, Lysosome	Cellular
1495	Interaction with the lung resident cell membrane components	Interaction with the lung cell membrane	Molecular
134	Increased, Activation and Recruitment of Hepatic macrophages (Kupffer Cells)	Increased, Activation and Recruitment of Hepatic macrophages (Kupffer Cells)	Cellular
55	N/A, Cell injury/death	N/A, Cell injury/death	Cellular
1492	Tissue resident cell activation	Tissue resident cell activation	Cellular
1493	Increased Pro-inflammatory mediators	Increased pro-inflammatory mediators	Tissue

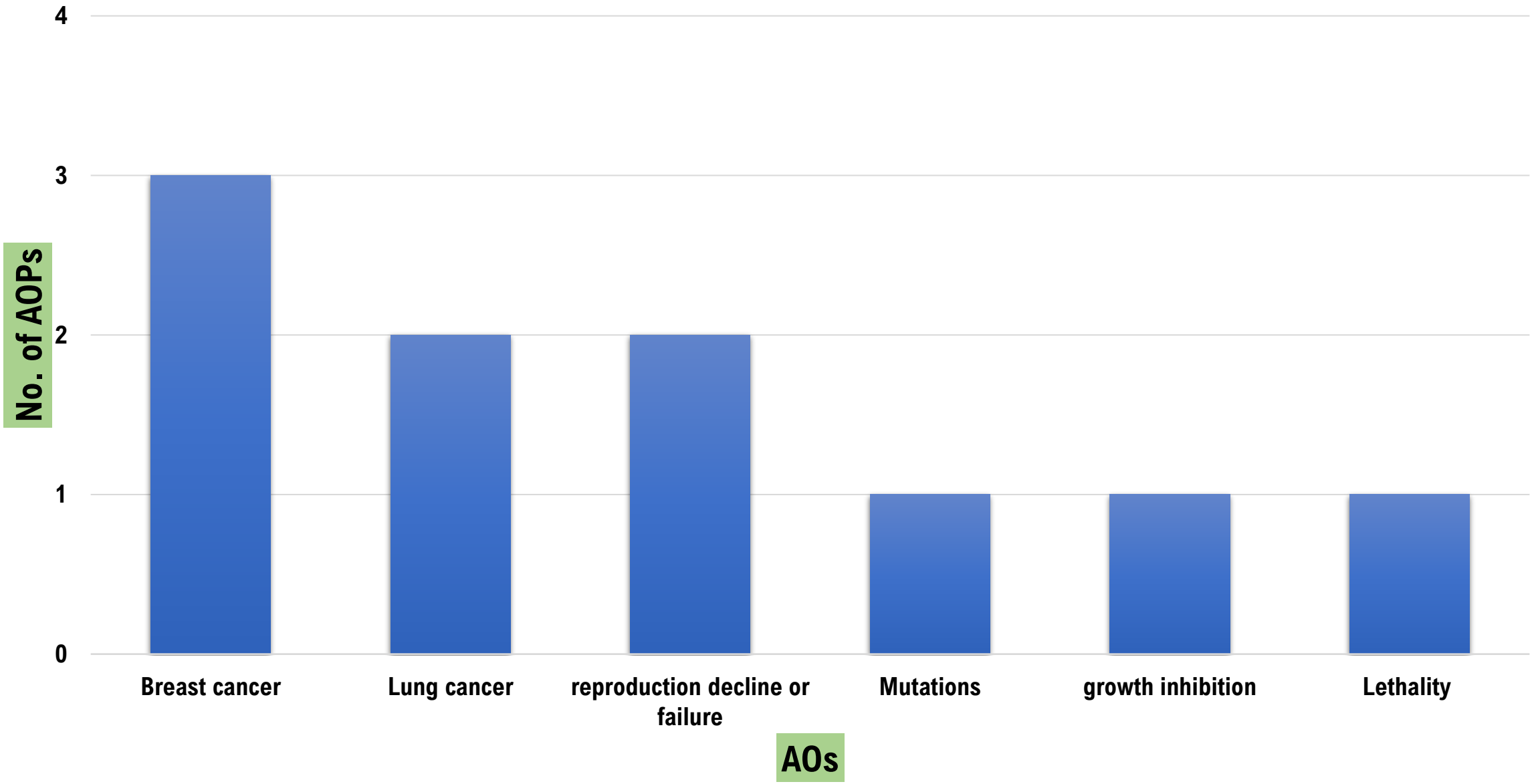
WIKI AOPs LINKED TO LYSOSOME DAMAGE



WIKI AOPs LINKED TO ROS FORMATION



WIKI AOPs LINKED TO DNA DAMAGE



SUMMARY: PART 2

Wiki AOPs that can be potentially explored for NMs

MIE/KE	No.of AOPs
CNT cellular interaction	1
Lysosome injury	14
ROS formation	12
DNA damage	10

Part 3: Generating testable AOPs for Nanomaterials

This project has received funding from the European Union's Horizon 2020 programme: grant agreement 814425.

GENERATION OF TESTABLE AOPs

Existing AOPs in AOP wiki → testable AOPs (with biological plausibility) for NMs using *in vitro* experiments

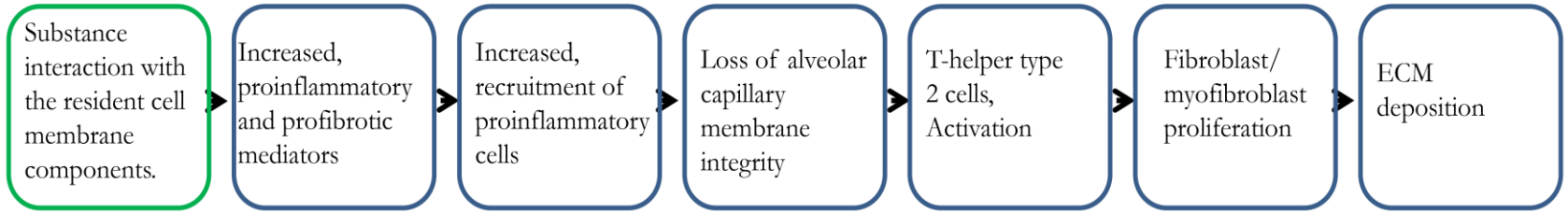
→simple (and linear) AOP

→To characterize intrinsic hazardous potential of a NM to induce an AOP

→potentially serve as a window to prioritize animal testing

CNT CELLULAR INTERACTION : Substance interaction with the lung resident cell membrane components leading to lung fibrosis (AOP 173)

AOP *in vivo*



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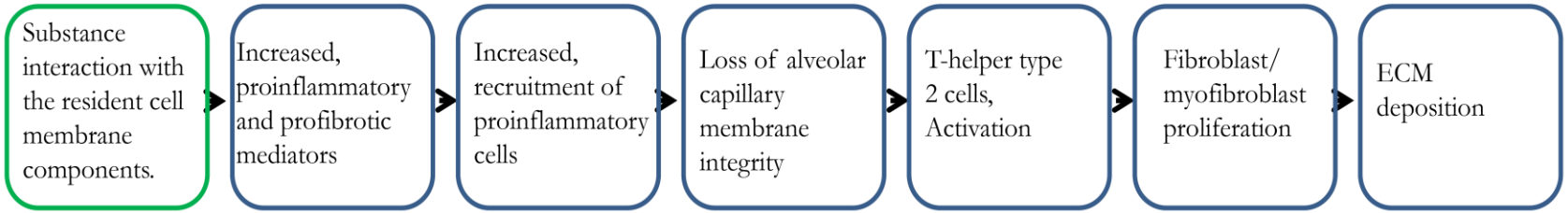
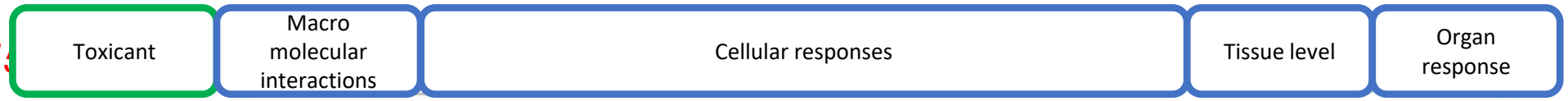
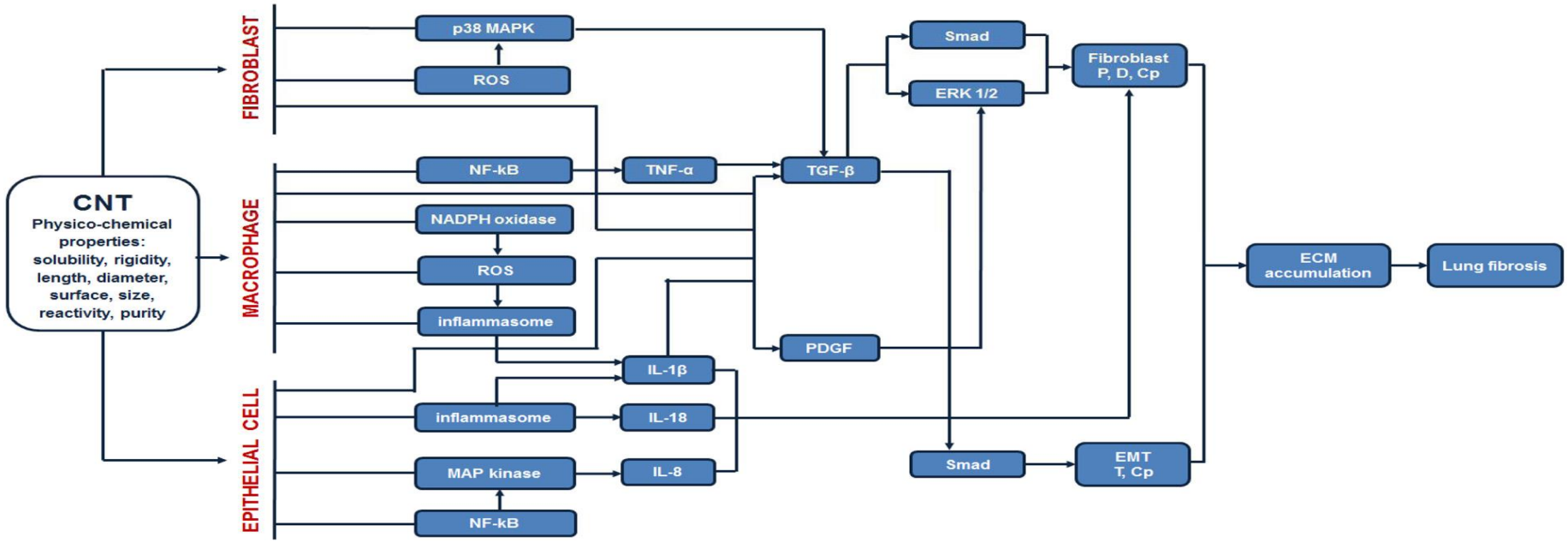


Consolidation of *in vitro* responses *(Vietti et al 201*



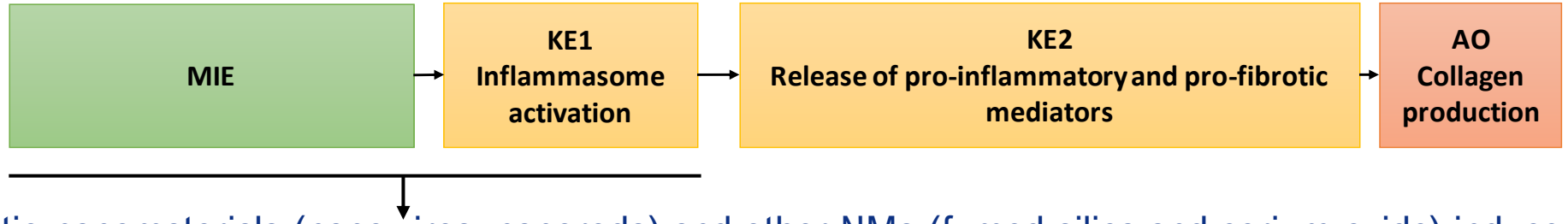


TESTABLE AOP FOR NM INDUCED LUNG FIBROSIS *IN VITRO*

AOP *in vitro*

TESTABLE AOP FOR NM INDUCED LUNG FIBROSIS *IN VITRO*

AOP *in vitro*



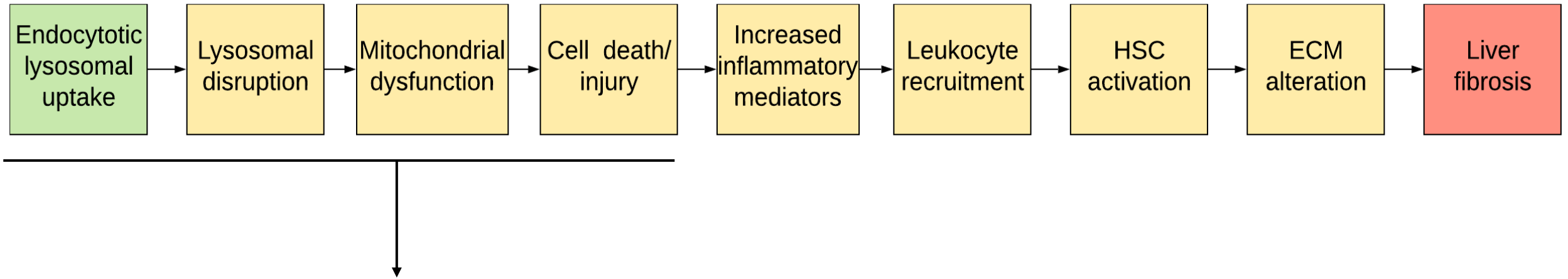
High aspect ratio nanomaterials (nanowires, nanorods) and other NMs (fumed silica and cerium oxide) induce inflammasome activation

(Wang et al 2017)

- *Lysosomal injury*
- *Membrane Perturbation*
- *Frustrated Phagocytosis*

LYSOSOME DAMAGE: Endocytic lysosomal uptake leading to liver fibrosis (AOP 144)

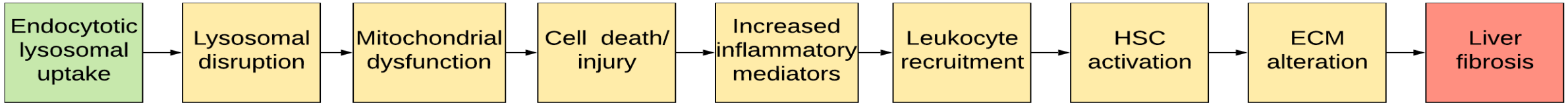
LYSOSOME DAMAGE: Endocytic lysosomal uptake leading to liver fibrosis (AOP 144)



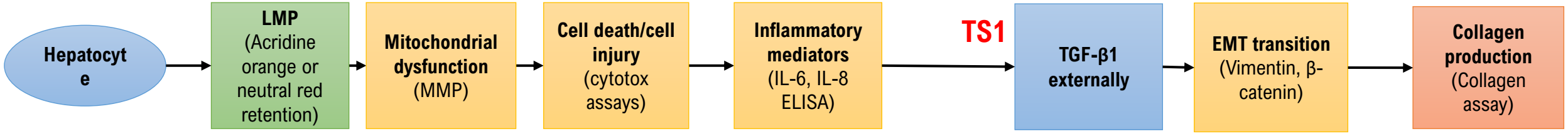
Lysosomal membrane permeabilization (LMP)- NM induced lysosomal disruption
→ recongnized as a death mechanism *(stern et al 2012)*

Slow LMP - apoptosis
Massive LMP - necrosis

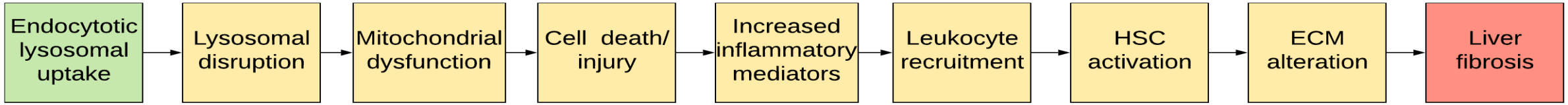
TESTABLE AOP FOR NM INDUCED LIVER FIBROSIS *IN VITRO*



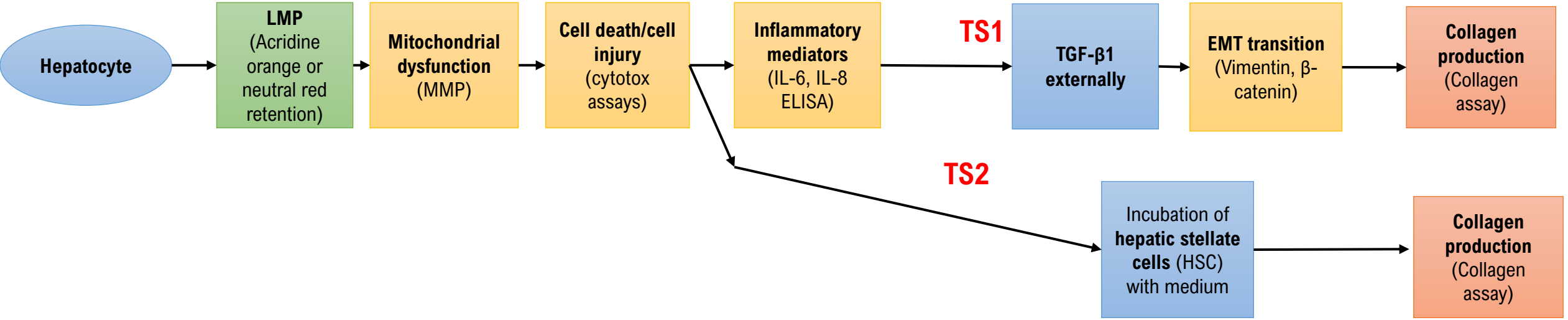
Testing strategies *in vitro*



TESTABLE AOP FOR NM INDUCED LIVER FIBROSIS *IN VITRO*



Testing strategies *in vitro*



TAKE HOME MESSAGE

→combining from existing AOPs in AOP wiki and existing knowledge (literature) - lot of potential to generate testable AOPs (*in vitro*) *for* NMs

Such strategy is useful

→to reduce animal testing in the long term;

- still require animal studies to obtain toxicokinetics information
- validate these *in vitro* AOPs

→to generate mechanistic information

- scientific

→to reduce the complexity of the experimental approach

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RISK GONE

THANK YOU!

Peter HOET, Professor
Sivakumar MURUGADOSS, Research Associate
KU Leuven
peter.hoet@kuleuven.be
sivakumar.murugadoss@kuleuven.be

www.riskgone.eu | riskgone@nilu.no

This project has received funding from the European Union's Horizon 2020 programme: grant agreement 814425.

Session 3: NanoQSAR-AOPs

Karolina **JAGIELLO**
Sabina HALAPPANAVAR Ulla VOGEL Tomasz PUZYN

This project has received funding from the European Union's Horizon 2020 programme: grant agreement 814425.

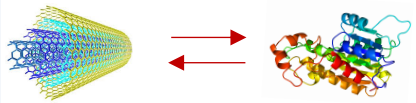
AOP for the risk assessment of nanomaterials
RiskGONE Webinar, 5 June 2020

Molecular interactions

Cellular and tissues effects

Organ effects

Substance interaction with cell components



Increased pro-inflammatory mediators

Recruitment of pro-inflammatory cells

...

Fibroblast/myofibroblast proliferation

Lung Response

- Molecular Initiating Event, MIE

- Key Events, KEs

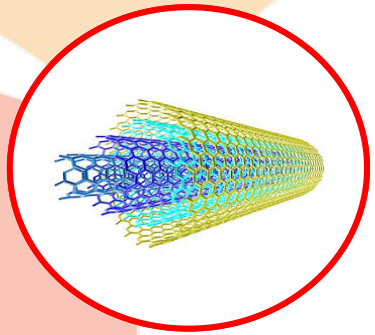
- Adverse Outcome, AO

Adverse Outcome Pathway

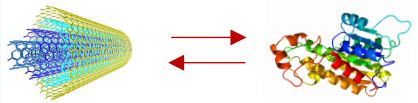
Molecular interactions

Cellular and tissues effects

Organ effects



Substance interaction with cell components



Increased pro-inflammatory mediators

Recruitment of pro-inflammatory cells

...

Fibroblast/myofibroblast proliferation

Lung Response

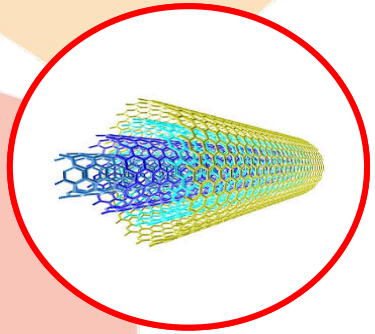
- Substance 'identities'
- Molecular Initiating Event, MIE
- Key Events, KEs
- Adverse Outcome, AO

Adverse Outcome Pathway

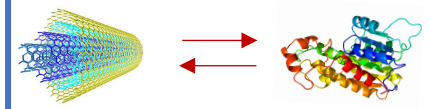
Molecular interactions

Cellular and tissues effects

Organ effects



Substance interaction with cell components



Increased pro-inflammatory mediators

Recruitment of pro-inflammatory cells

...

Fibroblast/myofibroblast proliferation

Lung Response

Biological events (e.g. MIE, KE) (**y**)

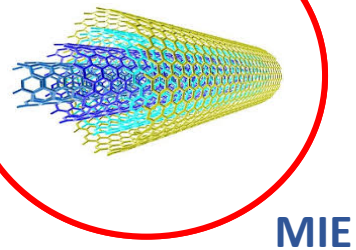
Structural features (**X**)

Which structural features are responsible for inducing biological events crucial for AO?

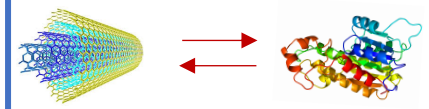
QSAR

Adverse Outcome Pathway

Molecular interactions | Cellular and tissues effects | Organ effects



Substance interaction with cell components



MIE = $f_{(\text{STRUCTURE})}$

Increased pro-inflammatory mediators

↓

Recruitment of pro-inflammatory cells

↓

...

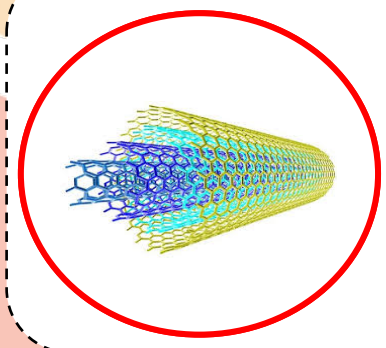
↓

Fibroblast/myofibroblast proliferation

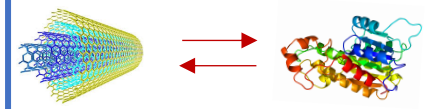
Lung Response

NanoQSAR-AOP

Molecular interactions | Cellular and tissues effects | Organ effects



Substance interaction with cell components



$KE = f_{(STRUCTURE)}$

Increased pro-inflammatory mediators

↓

Recruitment of pro-inflammatory cells

↓

...

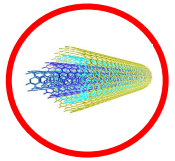
↓

Fibroblast/myofibroblast proliferation

Lung Response

NanoQSAR-AOP

NanoQSAR-AOP

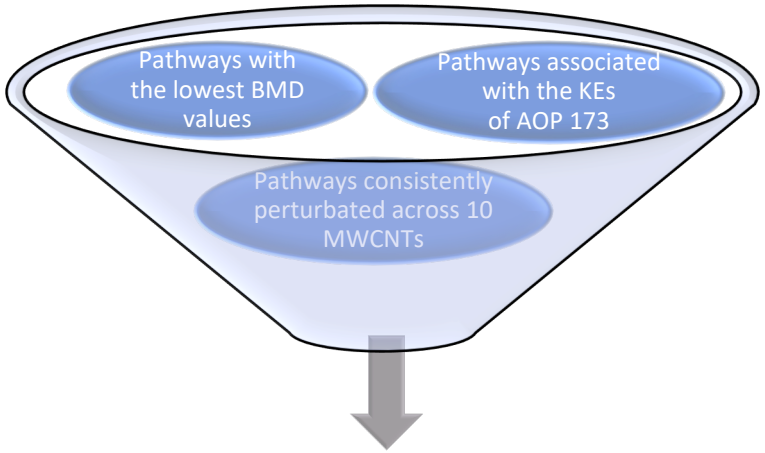


MIE

KE

$MIE/KE = f_{(STRUCTURE)}$

1. Selection of transcriptomic pathways for predictive model



Three individual pathways selected

Determination coefficient analysis

One pathway selected

2. Nano-QSAR model development

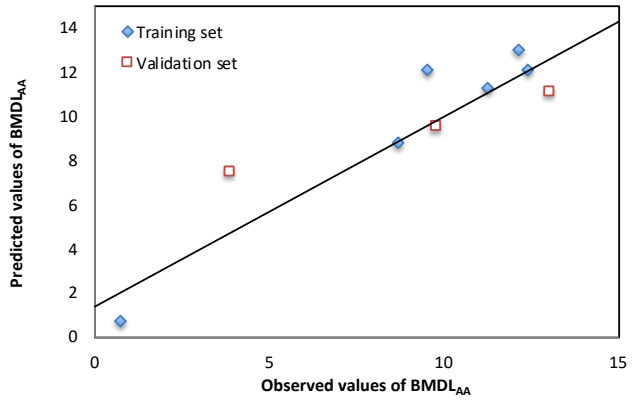
Structural properties:

BET Diameter OH

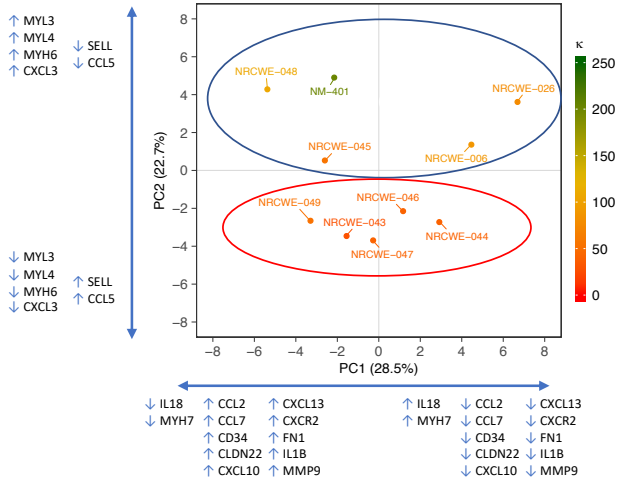
Length COOH κ

Transcriptomic pathways BMDLs

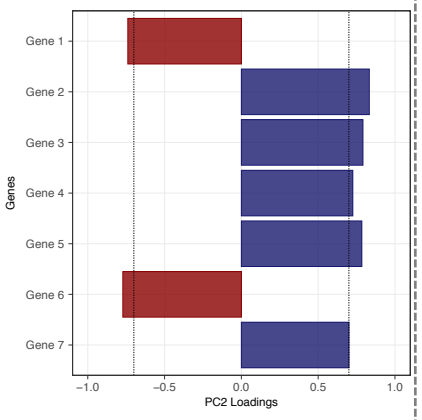
$BMDL_{transcriptomic\ pathway} = f(\text{structural properties of MWCNTs})$



3. Grouping MWCNTs based on the structure-activity relationships



Visualization of the MWCNTs in gene space associated with selected transcriptomic pathway and identification of genes relevant for PCs



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Jagiello et al (2020) submitted to Small

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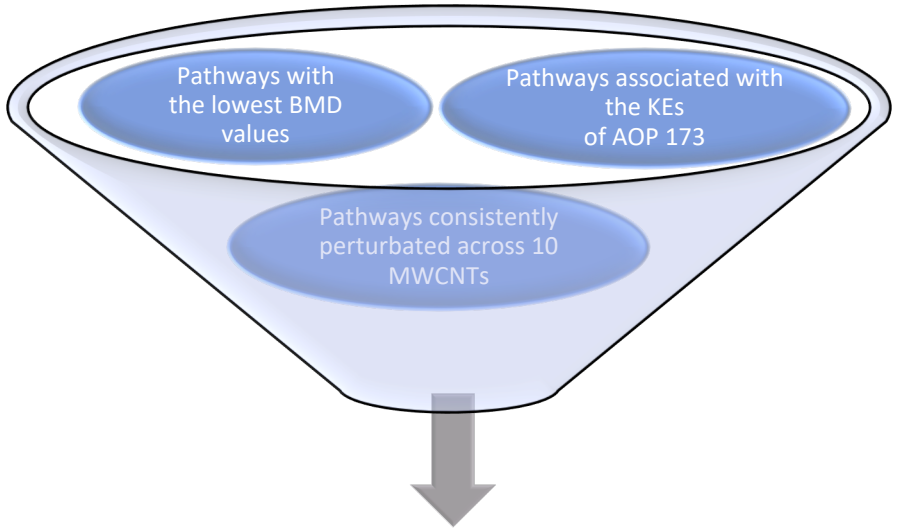
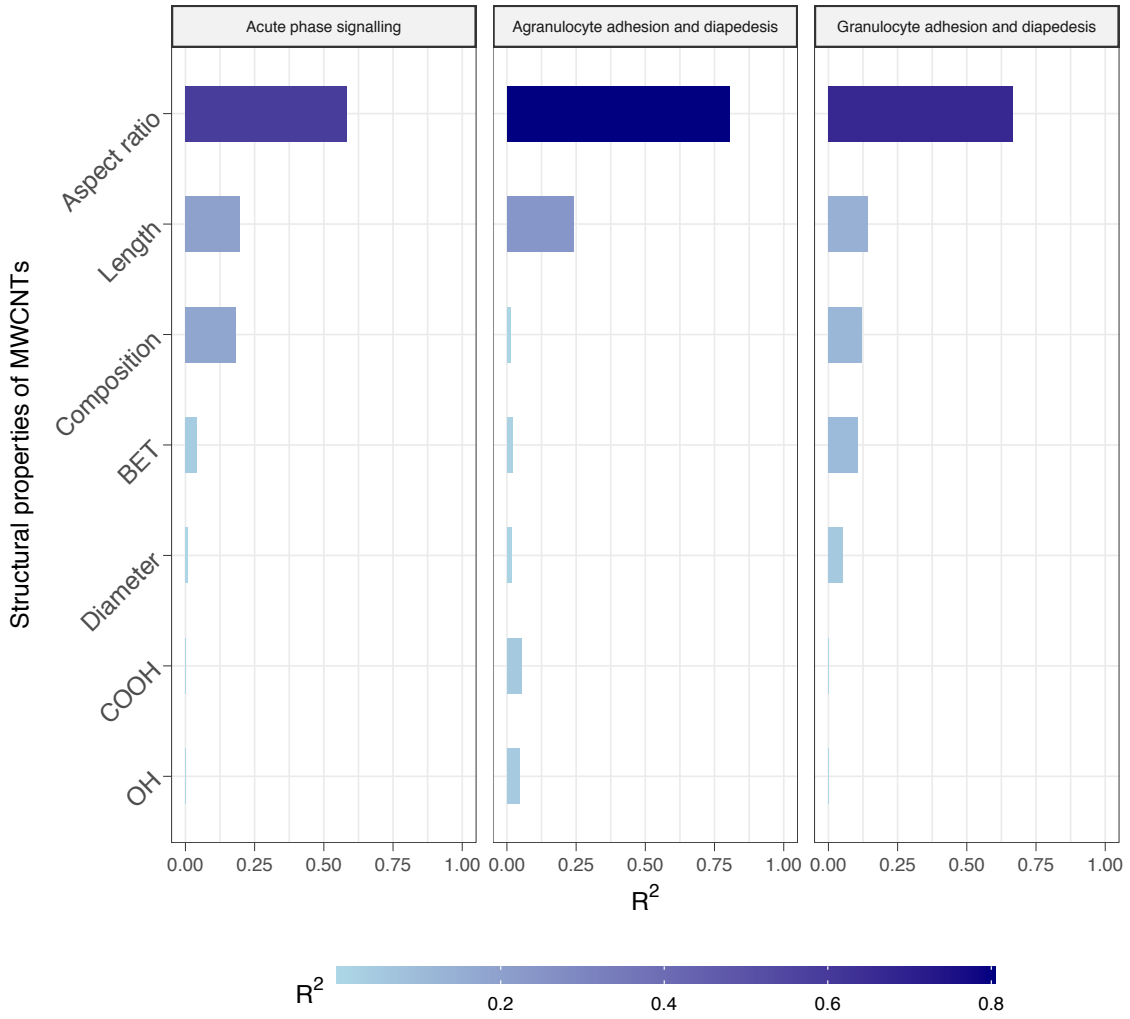


‚Agranulocyte adhesion and diapedesis'

‚Granulocyte adhesion and diapedesis'

‚Acute phase signaling'



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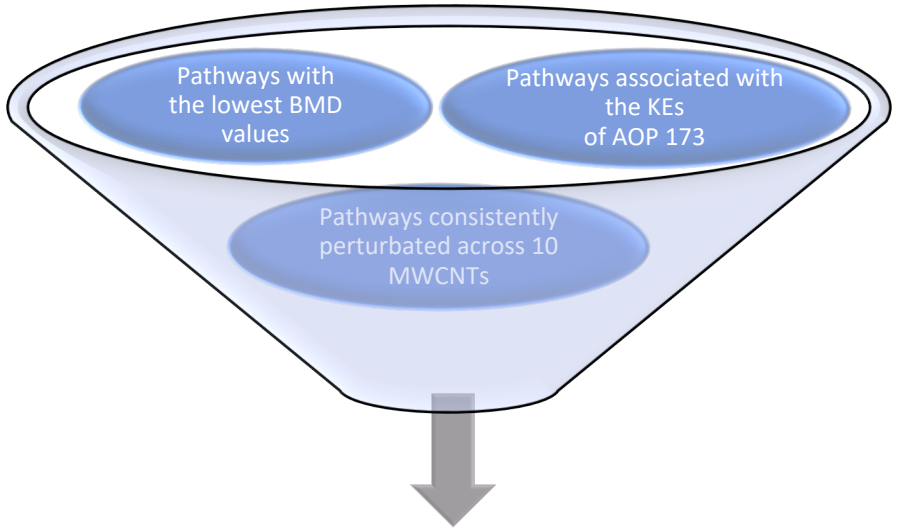

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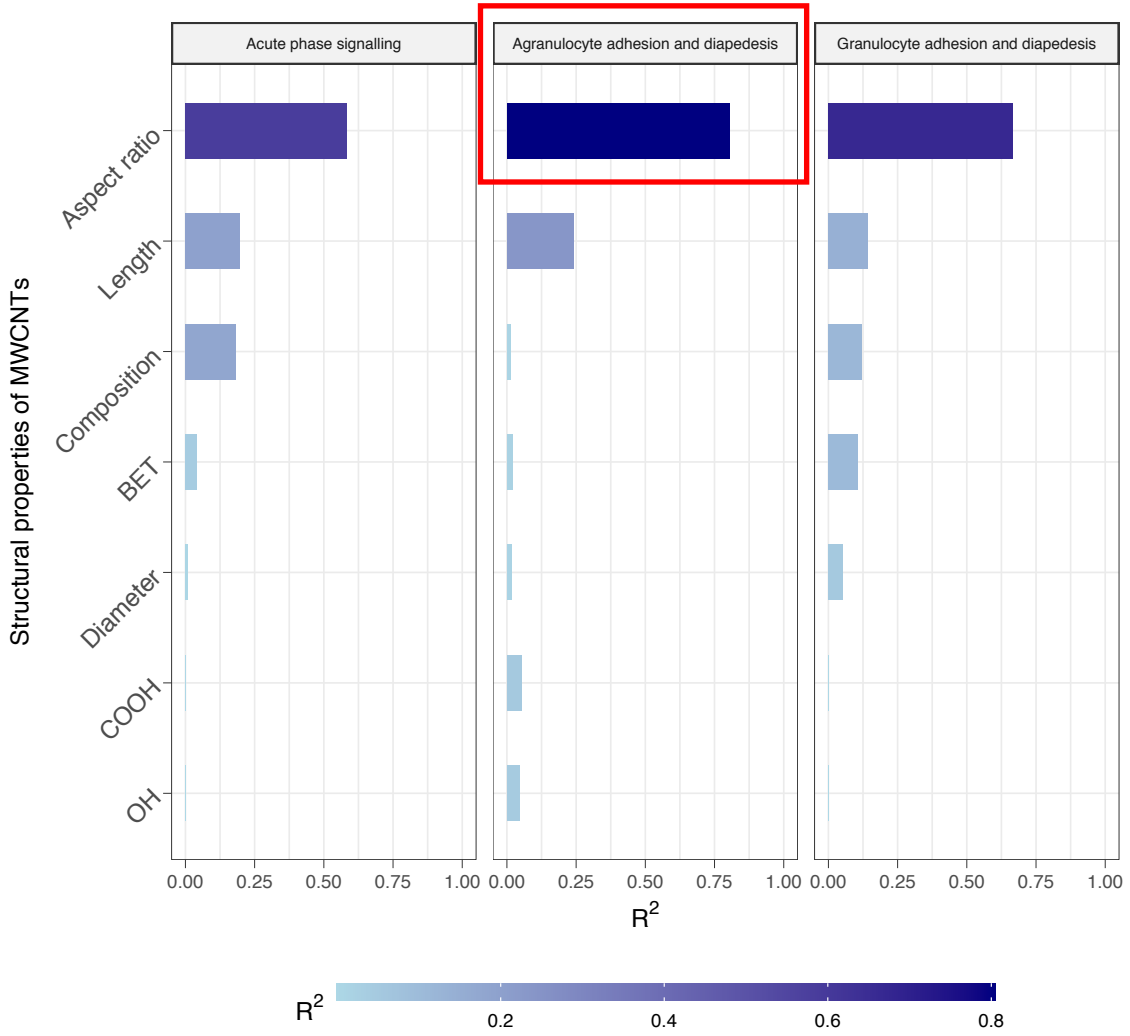

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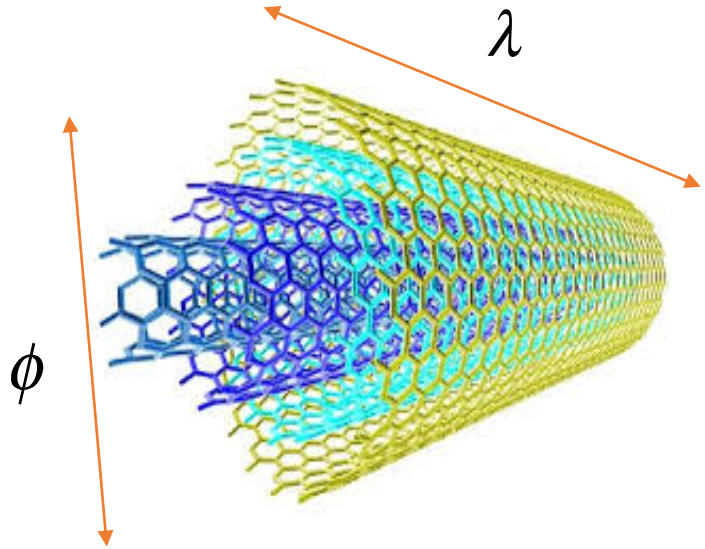

Aspect ratio (κ)

$$\kappa = \frac{\lambda}{\phi}$$

λ - length

ϕ - diameter

Agranulocyte Adhesion and Diapedesis

$$BMDL_{AA} = 15.07 - 0.07\,\kappa$$

$$R^2 = 0.86;\ RMSE_C = 1.63;\ Q^2_{EXT} = 0.62;\ RMSE_{EXT} = 2.34$$

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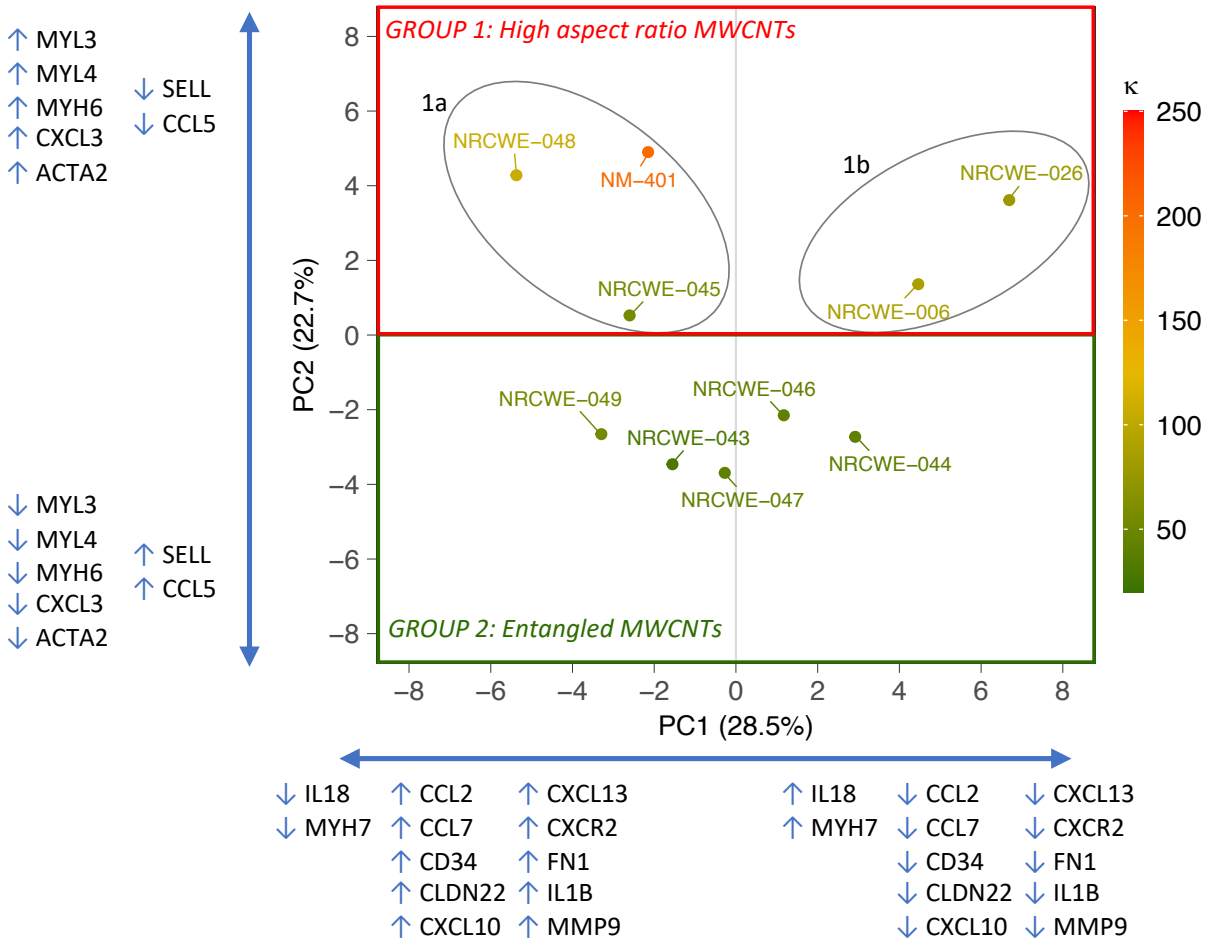
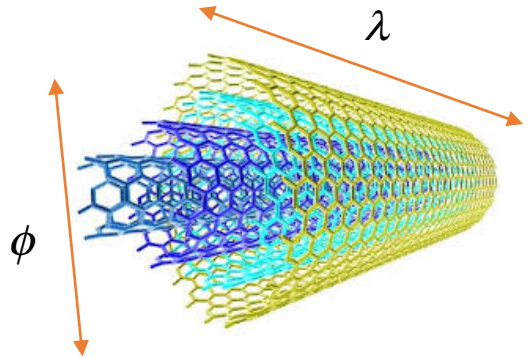
3. Grouping of MWCNTs based on the structure-activity relationships





$$PC2 = -7.54 + 0.11\,\kappa$$

$R^2 = 0.85$; $RMSE_C = 1.26$; $Q^2_{EXT} = 0.62$; $RMSE_{EXT} = 1.51$

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Advanced Tools for NanoSafety Testing

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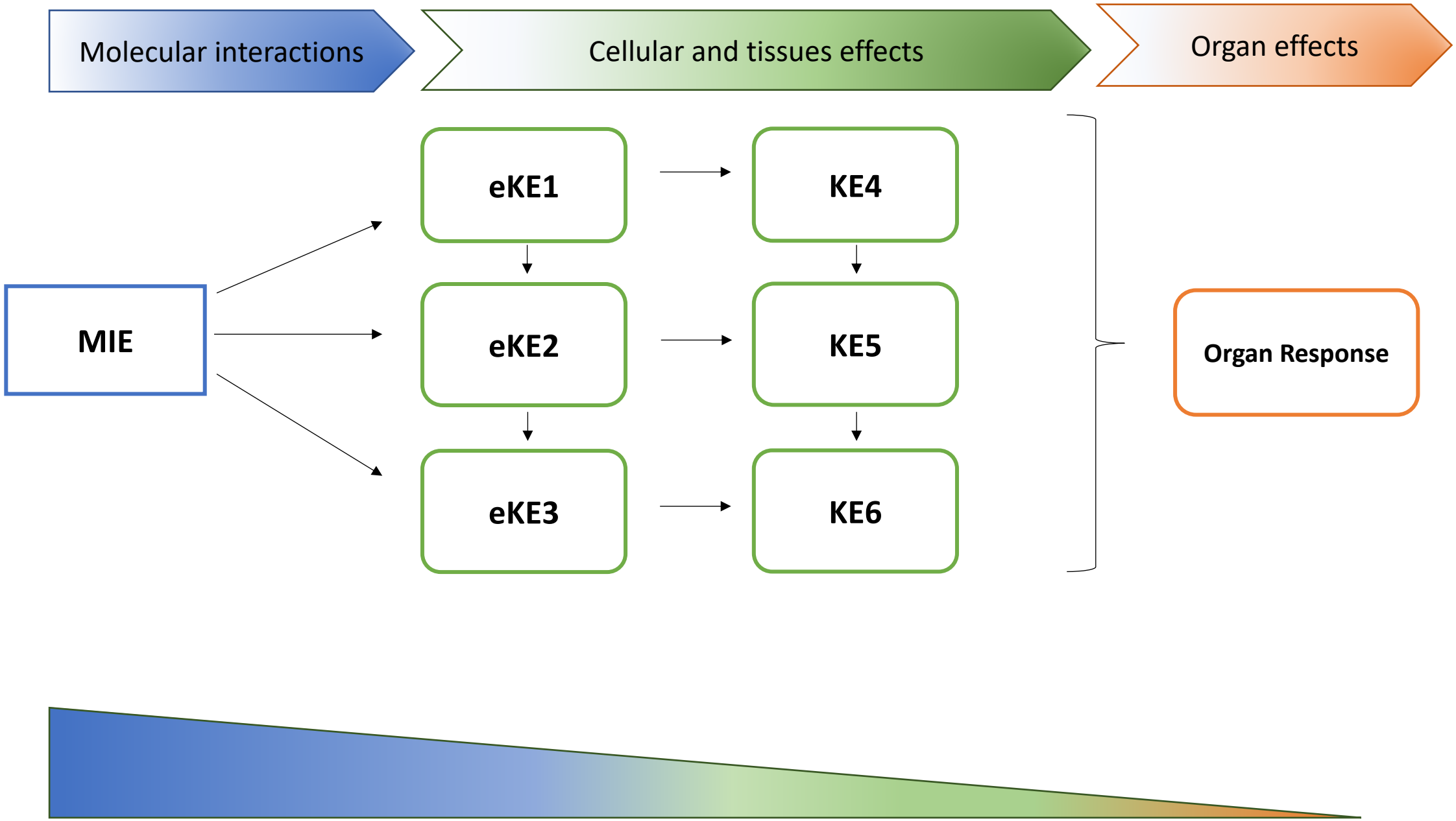


Selection of features specific for early biological changes that are essential for occurrence of AO

Molecular interactions

Cellular and tissues effects

Organ effects

MIE

eKE1

eKE2

eKE3

KE4

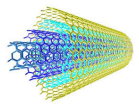
KE5

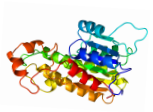
KE6

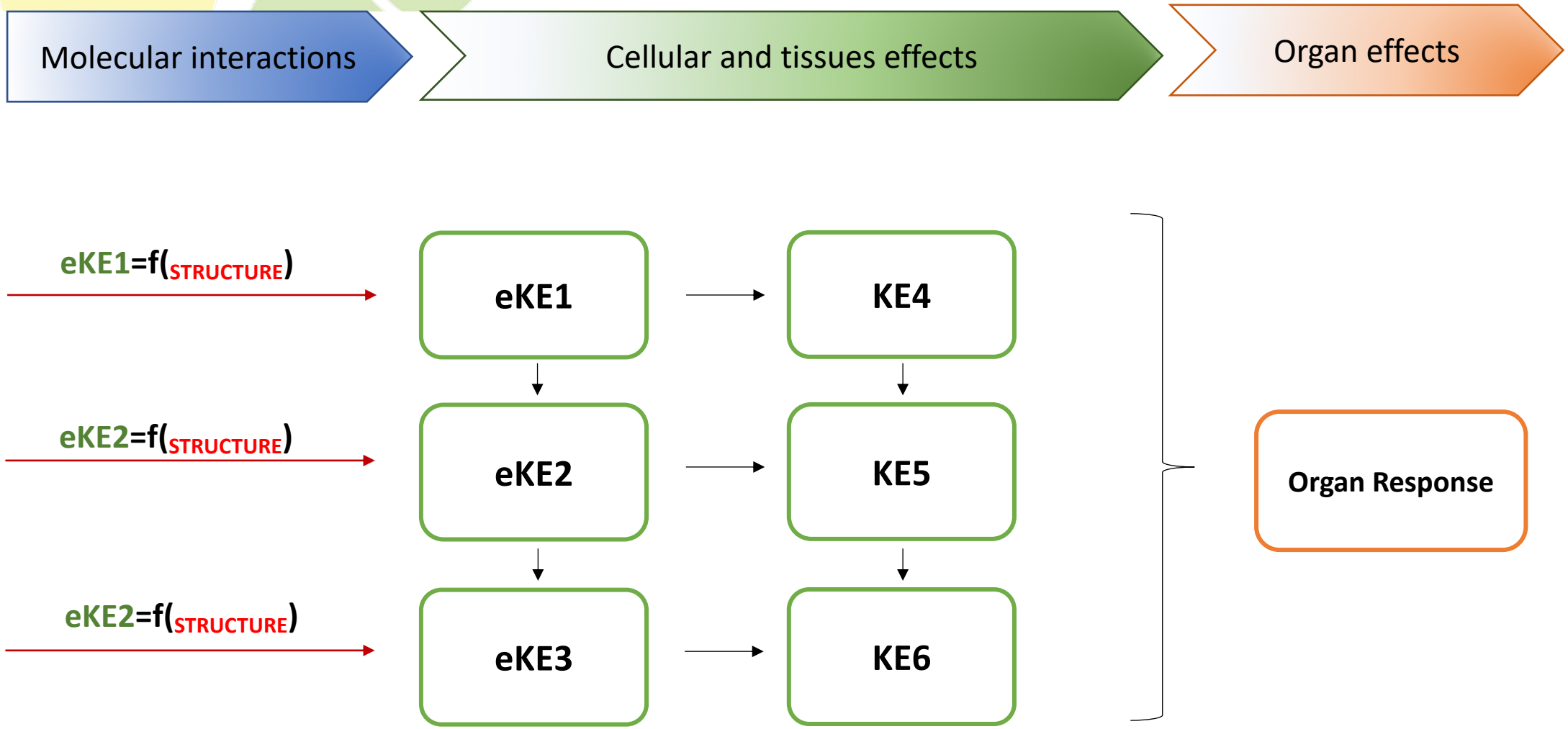
Organ Response

$BE_n = f(_{STRUCTURE})$

BE



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eKE1=f(STRUCTURE)
eKE2=f(STRUCTURE)
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eKE1
eKE2
eKE3
KE4
KE5
KE6
Organ Response

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THANK YOU!

Karolina JAGIELLO

QSAR LAB

k.jagiello@qsarlab.com

www.riskgone.eu | riskgone@nilu.no

This project has received funding from the European Union's Horizon 2020 programme: grant agreement 814425.